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APPLICATION OF RESPONSE SURFACE METHOD FOR LIPASE MEDIATED SYNTHESIS OF FATTY ACID AMIDE BIOSURFACTANT FROM DIETHANOLAMINE AND GLUCOSAMINE

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ABSTRACT

A fatty acid amide has been made from diethanolamine and glucosamine using Novozyme 435 lipase in organic solvents. Fatty acid amide is a surfactant that is widely used in the pharmaceutical and cosmetic fields. As a biosurfactant, fatty acid amides have the advantage of being a surfactant that is renewable and environmentally friendly. This study aimed to obtain biosurfactants by applying the Response Surface Method and determining the effect of adding lipase, amine, and the yield of fatty acid amide. The results showed that the addition of amines and temperature would increase the yield of the two amides produced. However, the effect of temperature is more significant than the addition of amines. The optimal results obtained in the synthesis of diethanolamide were obtained when using enzyme 11-12%, diethanolamine 2-2.5 molar, and temperature 45-49ºC, and in the synthesis of glucosamide, optimal glucosamide yield was obtained when using enzyme 9-10%, glucosamine 2-3 molar, and a temperature of 40-45ºC.

Keywords: glucosamine, diethanolamine, central composite design, fatty acid amide.

INTRODUCTION

The study of biosurfactants helps produce products with properties suitable for the desired application. Biosurfactants are most widely used in products directly related to the human body, such as food, cosmetics, and medicines. However, biosurfactants in the food and non-food industries are still not competitive due to the high production costs. However, biosurfactants' production and application are spurred to grow because of environmental problems caused by synthetic surfactants. Therefore, it is necessary to find a more economical production process so that biosurfactants can compete with chemical surfactants [1,2,3].

Most biosurfactants are produced by microorganisms such as bacteria, yeast, and molds using cell biotransformation. Surfactants produced by several microbes will grow on a variety of different substrates, from carbohydrates to hydrocarbons. Changes in the substrate also change the chemical structure of the product to change the properties of the resulting biosurfactant. Some microorganisms also exist that produce enzymes, where this enzyme can be used as a catalyst in condensation, acylation, esterification, hydrolysis, and alcoholysis. Thus these processes are used to manufacture various surfactant products including monoglycerides, phospholipids, and amino acid surfactants [4]. Studies on the production of biosurfactants by fermentation and biotransformation must be carried out to obtain high yields, using cheap or even non-selling raw materials. One of the strategies for producing biosurfactants is to use raw materials from the agricultural industry and their byproducts, including the waste they produce [5,6].

Microorganisms have long been used to produce emulsifiers and biosurfactants and aid in fat solubility. Hundreds of enzymes are known for their specificity for various substrates, but only a few are isolated in their pure form and crystallized, and only a few are known for their structure. The advantage of using protein in biotechnology has made the enzyme industry necessary. For example, proteases and lipases used in the detergent industry, amylase, and glucose isomerization are used in the starch industry or the synthesis of other organic compounds [7,8,9].

Lipase can be an option as a catalyst in the food, detergent, pharmaceutical, and cosmetic industries. Lipase (*triacyl-glycerol ester hydrolase*, EC 3.1.1.3) is part of the hydrolysis enzymes that can attack carboxylic bonds, such as hydrolyzing triglycerides, diglycerides, monoglycerides, fatty acids, and glycerol. The catalytic efficacy of several types of commercial lipases, namely fungal lipase, yeast lipase, bacterial lipase, and mammalian lipase for the condensation reaction of vinyl carbonate with phenyl-ethyl amines, indicates that yields of more than 70% yield will be obtained if using Rhizomucor miehei lipase, SP524 and lipase B from Candida Antarctica, Novozym 435 [10,11,12].

Dodecylic acid was chosen for use in the synthesis of fatty acid amides. Dodecylic acid is one of the three most common saturated fatty acids, for example, in cinnamon oil (80-90%), coconut oil (40-60%), and palm kernel oil (40-50%). This acid is used in the pharmaceutical industry because of its good antimicrobial properties. Dodecylic acid is also widely used in the manufacture of shampoos, soaps, cosmetics, and other surface-active ingredients. Various derivative forms of fatty acids can be obtained, among others, informing polyols with fatty acid esters such as sorbitol, glycerol, and sucrose [13].

Fatty acid amide from diethanolamine, namely alkyl dietanolamide, is one of the essential fatty acid

amide surfactants. Diethanolamine is a compound consisting of an amine and a di-alcohol group. The alcohol shows two hydroxyl groups on the molecule so that the resulting fatty acid amide surfactants are expected to have advantages over the available surfactants and are more environmentally friendly [14,15].

This surfactant serves as a stabilizer and foam developer. This is because oily dirt such as sebum causes the foam's stability, liquid soap, or shampoo to be drastically reduced. To overcome this, a foam stabilizer is needed, which functions to stabilize and change the foam structure to obtain more foam, thick with less foam. In soap making, diethanolamide is used to make the soap soft. The use of diethanolamide in shampoo formulas can prevent removing excess oil from the hair (excessive fatty effect). The resulting product does not cause pain in the eyes, making it suitable for soap and shampoo products for babies [16,17].

Fatty acid amide from glucosamine is obtained from the reaction between fatty acids, fatty acid methyl esters, or triglycerides with glucosamine. Glucosamide is widely used as a pharmaceutical and other biochemical product. This surfactant belongs to the alkyl-glucamide surfactant group, where this surfactant group is produced in large quantities as a cleaning agent. An example is N-dodecanoil-N-methyl glucamide. These surfactants use raw materials from the amine sugar group. Amine sugar compounds play an essential role in the formation and repair of cartilage. The amine sugar compounds' mechanism of action is to inhibit the synthesis of glycosaminoglycans and prevent cartilage destruction. Amine sugar can stimulate cartilage cells to form proteoglycans and collagen, which are essential proteins to improve joint function [18].

The application of biotechnology to surfactant synthesis has recently received significant attention. Biotechnology can be defined as using living bodies and biological/chemical processes in a metabolic process to produce higher economic value products. In line with the above definition and supported by the amount of vegetable oil as a raw material supplier for biosurfactants, the application of biotechnology in the synthesis of biosurfactants has the excellent potential [19].

From the studies that have been carried out, it is necessary to continue studying the synthesis of fatty acid amides using raw materials of diethanolamine, glucosamine, and dodecylic acid, using an organic solvent tert-amyl alcohol, and immobilized catalyst Novozyme 435. The use of the response surface method (RSM) was also chosen for optimizing the yield of the two synthesized surfactants.

MATERIALS AND METHODS

Enzymes and Chemicals

The chemical and biological substances used in the synthesis of fatty acid amide surfactants are dodecylic acid, diethanolamine, glucosamine from E Merck. Tert-amyl alcohol, isopropanol, and analytical materials, namely KOH, Phenolphthalein, Acetone, Methanol, Tri fluoro acetic acid (TFA) from E Merck. The immobilized enzyme Novozym 435 (lipase type B from *Candida Antarctica* bound by acrylic resin, activity 7000 PLU/G at 60^{o}C) was obtained from Novo Nordisk Industries (Denmark).

Response Surface Methodology

Several parameters affect the condensation reaction, but the amount of catalyst, the amount of amine, and temperature were chosen as the parameters that most influence the condensation reaction. The RSM is used to evaluate reaction parameters' effect and optimize the response [20, 21]. The Central Composite experimental design with three factors and five levels was used. The design consisted of 20 experimental runs with six replications at the center point [22].

Experimental design and response were made using the Design Expert 10 program, with three independent variables, namely the amount of enzyme (A,%), the amount of diethanolamine/glucosamine (B, Molar), and temperature (C, ^{o}C). Determination of the minimum and maximum limits of the independent variables obtained from previous studies and trial error. Furthermore, the lower and upper limit values are entered into the program for combination randomization so that the experimental design will be analyzed as in Table-1. The response to be measured and optimized is the yield of fatty amide.

Synthesis and Analysis

Dodecylic acid and diethanolamine were placed in capped reaction vials. Tert-amyl alcohol was added to the reaction mixture and stirred until the reactants dissolved. Novozyme 435 was then added into the reaction vials with the ratios arranged in the experimental design in Table-1. The reaction was conducted with agitation at 45^{o}C for 12 h as long as the stirring reaction continues and the temperature is maintained constant. Then the product mixture is separated from the enzyme using a vacuum filter, and tert-amyl alcohol was removed using a rotary evaporator. The product formed is taken and washed with acetone so that it is clean from residual amines. The above steps are repeated for reaction temperatures of 50 and 55^{o}C. The same synthesis was carried out by replacing diethanolamine with glucosamine. According to the experimental design in Table 2, the condensation reactions were carried out in the capped reaction vials at 40^{o}C during 24 h in the presence of Novozym 435.

FTIR Analysis

Functionalization of the purified product was analyzed by Fourier transform infrared spectroscopy (Shimadzu FT-IR) in the range from 400 to 4000 cm^{-1}.

HPLC Analysis

Analysis of dodecylic acid, amides, and esters amide was carried out using the Perkin Elmer HPLC Series 200 system on the Silica C-18 column, 4 μm, a wavelength of 280 nm, a flow rate of 1 mL/minute, a

pressure of 2200 psi and a temperature of 440ºC, using the mobile phase. methanol: water: TFA (80:20:0.3 v/v/v). The product composition was analyzed by comparing the difference in retention times between raw materials and fatty amide products.

RESULTS AND DISCUSSIONS

This study aimed to obtain a high yield of fatty amides through the experimental design of the Central Composite Design (CCD) and using RSM. The independent variables used are the enzyme amount, molar of amine, and temperature, while the response to be measured and optimized is the fatty amide yield.

Table-1. Experimental design of the dietanolamine condensation reaction and yield response.

Run	A-Enzyme (%)	B-Diethanolamine (Molar)	C-Temperature (ºC)	R1-Yield of Diethanolamide (%)
1	8	4	45	87.575
2	10	3	50	89.615
3	10	3	41.591	92.905
4	10	4.681	50	76.945
5	8	2	45	97.310
6	12	2	55	97.985
7	12	4	55	68.600
8	8	4	55	35.815
9	10	3	50	17.815
10	6.636	3	50	64.995
11	12	2	45	97.740
12	10	3	50	85.605
13	8	2	55	96.960
14	10	1.318	550	98.415
15	10	3	58.409	41.845
16	13.364	3	50	93.490
17	10	3	50	94.905
18	12	4	45	92.100
19	10	3	50	42.725
20	10	3	50	67.440

Table-2. ANOVA results for response surface quadratic model for diethanolamide synthesis.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	6332.21	9	703.58	1.37	0.3149
A-Enzyme	550.26	1	550.26	1.07	0.3253
B-Diethanolamine	1476.75	1	1476.75	2.87	0.1210
C-Temperature	1903.62	1	1903.62	3.70	0.0832
AB	160.70	1	160.70	0.31	0.5884
AC	104.08	1	104.08	0.20	0.6624
BC	706.03	1	706.03	1.37	0.2684
A^2	469.49	1	469.49	0.91	0.3618
B^2	1088.49	1	1088.49	2.12	0.1763
C^2	32.94	1	32.94	0.064	0.8053
Residual	5142.08	10	514.21		
Lack of Fit	499.70	5	99.94	0.11	0.9856
Pure Error	4642.38	5	928.48		
Cor Total	11474.28	19			

So far, fatty acid amides are often synthesized using a chemical ZnO catalyst at a temperature of 150^{o}C for 6-12 hours. The use of a chemical catalyst requires a step of protection and deprotection of the hydroxyl group to prevent amines' carbonation with CO_2. The use of this high temperature also results in an unattractive color in the final product. For this reason, the fatty acid amide synthesis is carried out using a biocatalyst that can work at lower temperatures so that it requires less energy, and the final product is brightly colored [9, 11].

Diethanolamine is used as a source of amines with a better degree of polarity because it has two hydroxyl groups in its molecule. Glucosamine, which is also used as a source of amines, is a vital amine sugar compound. Amine sugars can be obtained from the reaction of glucose, lactose, or other sugars with ammonia or alkyl amines.

Dodecylic acid with the two types of amines used has a different polarity. Amine is only slightly soluble in hydrophilic solvents, while dodecyl acid dissolves well. There-amyl alcohol is chosen to be used as a solvent because it can dissolve diethanolamine and glucosamine. This organic solvent is non-toxic and is not a lipase substrate. The immobilized lipase catalyst from Candida Antarctica was chosen because this immobilizing enzyme is easy to obtain, stable in solvents, and easy to recover [18].

Response of Yield on the Synthesis of Diethanolamine

The combination of the three variables was randomized to get 20 treatments at the RSM Central Composite Design. A combination of treatments was performed, and the percent yield analysis of diethanolamide was carried out using HPLC. Experimental results that cover each yield response to fatty acid amides, namely diethanolamide, are then tested on analysis of variance (ANOVA) to determine the recommended model and test the significance of interactions between variables response [23].

The ANOVA results for the response surface quadratic model for the synthesis of diethanolamide are given in Table-2. The recommended model is selected by referring to the ANOVA result, which gives the most considerable R^2 value (0.5519). From Table-2, it is found that the model F-value of 1.37 shows that the model is less significant relative to the noise. There is a 31.49% chance that an F-value this large could occur due to noise. The desirability value (df) is a function value that shows the program's ability to fulfill the desires based on the specified criteria. Suppose the value of DF is close to one. In that case, it means that combining the three selected variables to produce the desired diethanolamide yield response is more perfect [24].

The model chosen to analyze the variables is a model that gives significance to ANOVA and gives non-significance to the lack of fit. For that, the response variable can be related to the following polynomial equation,

$$Yield = +66.17 + 6.35A - 10.40B - 11.81C + 4.48AB + 3.61AC - 9.39BC + 5.71A^2 + 8.69B^2 + 1.51C^2 \quad ...(1)$$

where A is the amount of enzyme (%), B is the molar of diethanolamine (M), and C is the temperature (oC).

Besides that, a normal residual plot is carried out as shown in Figure-1, where this plot is used to indicate whether the residual follows a normal line (straight line). Figure-1 shows that the residuals do not follow the normal

line. The residual is the difference between the actual response and the predicted response value.

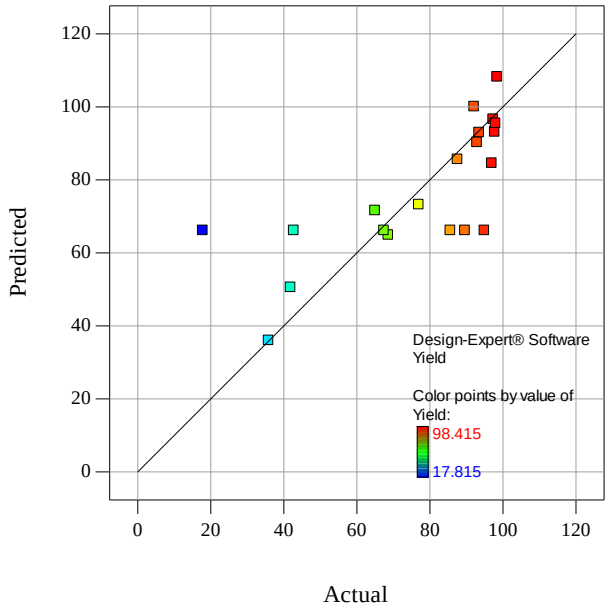


Figure-1. The normal plot of diethanolamide synthesis residuals.

If the data points get closer to the standard line, this indicates that the data is spread commonly, and the actual results will be closer to the results predicted by the model [25].

The amount of amine used for the condensation reaction is one of the most critical parameters affecting the fatty acid amide yield. At a 1:1 ratio, the ratio of fatty acids to amines is the minimum required reaction. However, condensation is a reversible reaction. Using high amounts of amines is always advantageous to obtain maximum yield [15]. To obtain the optimal molar ratio of amine/fatty acid, a condensation reaction is carried out using 2 to 4 molar of diethanolamine per molar of dodecylic acid.

To optimize the interaction between the enzyme and molar amounts of diethanolamine on the response in the form of diethanolamide yield, the surface response and contour plots were obtained, as shown in Figure-2.

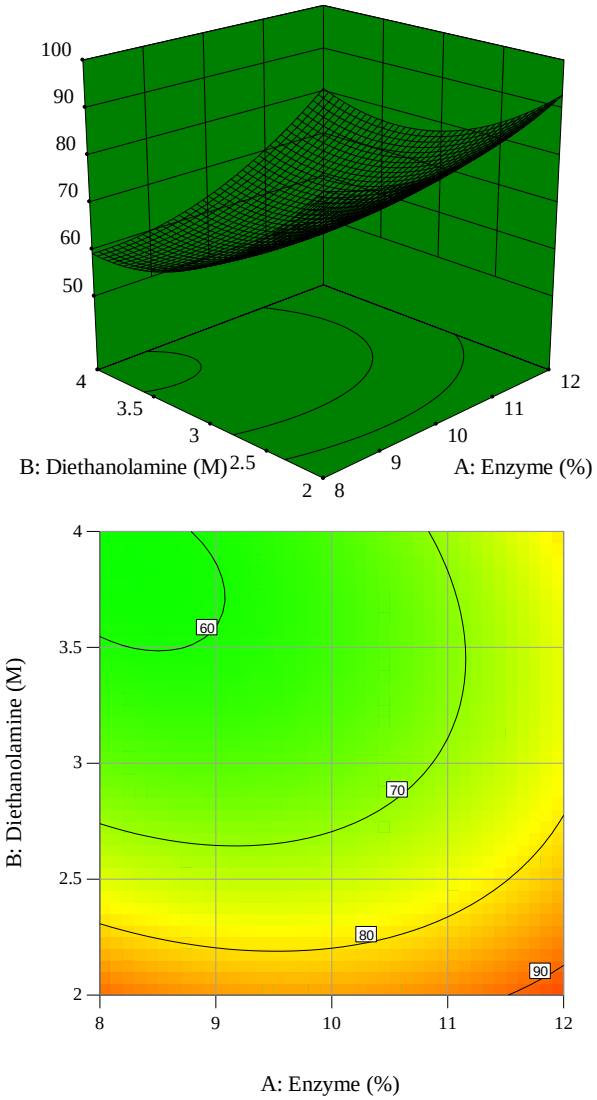


Figure-2. Surface response and interaction contours of the amount of enzyme and molar amine on dietanolamide yield.

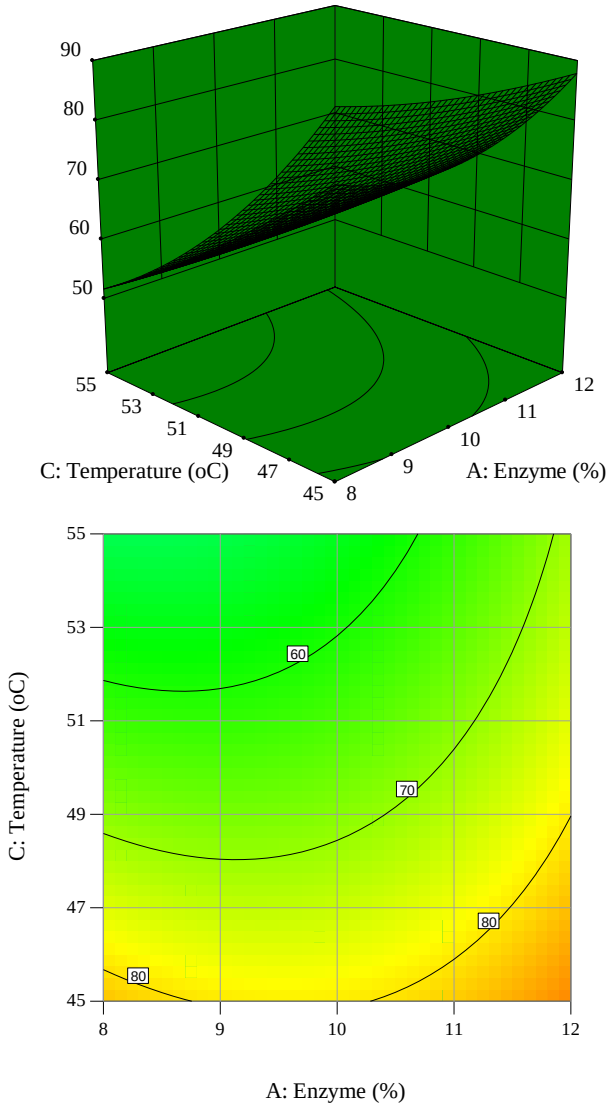


Figure-3. Surface response and interaction contours of temperature and enzyme amount on dietanolamide yield.

A maximum yield of 90% was observed for the maximum enzyme use of 12%, but the minimum amount of diethanolamine was 2-2.5 molar. The amide yield appears to increase linearly with the increase in the number of enzymes. In contrast, the addition of molar diethanolamine reduces the yield of amines, and a minimum yield of 60% is obtained precisely when diethanolamine from 3.5 to 4 molar is used. This is because, during synthesis, free amines interact with lipases [9]. The number of acids decreases as the reaction progresses until the reaction is equilibrated. In this state, the acid number will be much reduced, which means that amines' conversion to amides has been optimal.

From the table of the results of diethanolamide acquisition in Table-1, it can be seen that there is an effect of increasing the concentration of diethanolamine. The function of enzymes is as a catalyst. The more Novozyme 435 catalysts are used, the more fatty acid amides will be produced. This is due to the large number of catalysts used.

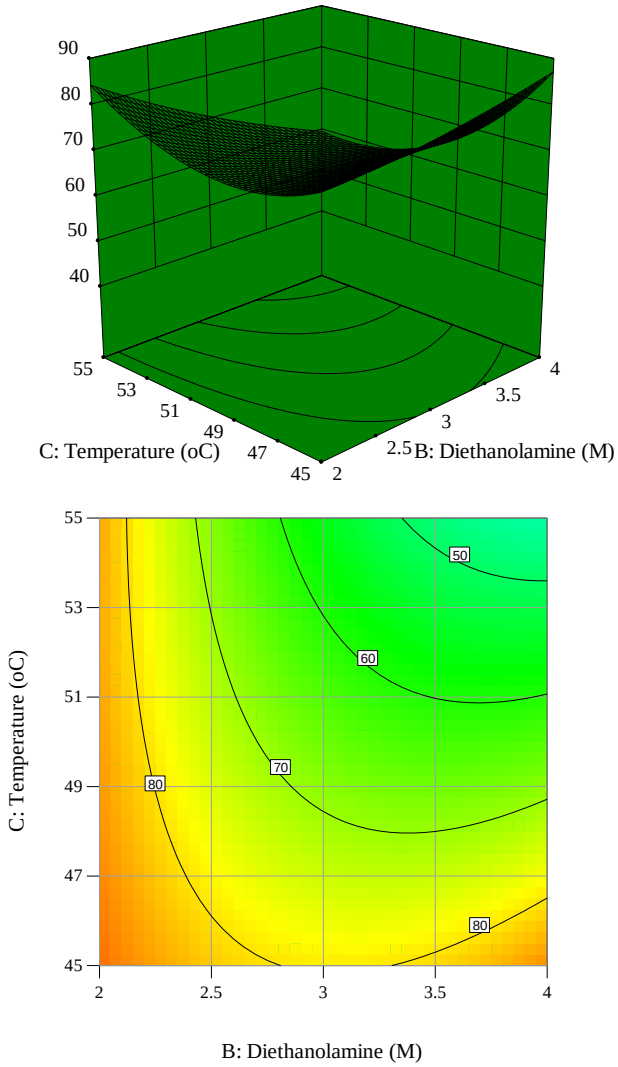


Figure-4. Surface response and interaction contours of temperature and molar amine on dietanolamide yield.

The faster the reaction occurs so that, the faster the surfactants are formed [14].

The cost of enzymes is an essential factor in determining the economics of the process. Enzymes with high stability and high recycling possibilities are highly desirable. Research on the epoxidation of toluene using enzymes found that the enzyme's efficiency was 75% after 15 reaction cycles [5, 16]. However, if the enzyme is operated in the solvent-free process at optimum conditions, the enzyme loses much of its activity, thus limiting the amount of recycling. In-depth knowledge of the factors that cause deactivated enzymes is essential in preparing lipases with enhanced stability for optimal process design.

Lipase enzymes can work well in organic solvents [6]. Therefore, the organic solvent tert-amyl alcohol (log P=1.5) was chosen for the synthesis. Enzyme concentration dramatically affects the rate of reaction and

the quantity of the product. In this study, 8-12% of the immobilizing enzyme was dissolved in the reactor, and a 60-90% yield of diethanolamide will be obtained.

The temperature variable and its interaction with the amount of the enzyme influence the amide recovery. The interaction between the two is observed at a constant three molar amount of diethanolamine, as shown in Figure-3. An increase in enzyme use from 8-10% does not appear to increase amine yield, but if more than 10% enzymes are used, an increase in the amount of enzyme and temperature will simultaneously increase amide yield. The surface response and contour plots in Figure-3 show that the maximum yields are in the range of enzymes 10 to 12% and 45-49ºC.

The further increase of the catalyst concentration did not increase the reaction yield significantly. If the catalyst concentration is enlarged, the methyl ester bonds will be broken more efficiently so that RCO + will be formed more quickly, which means that the resulting fatty acid amide will increase. This condensation reaction's byproduct is water, which is formed because OH- ions will bind hydrogen ions from dodecylic acid and form water [10].

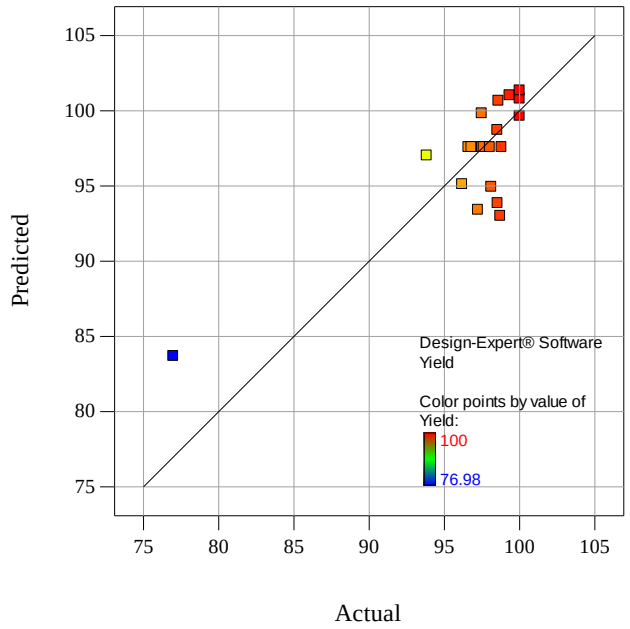


Figure-5. The plot of the normal residuals of glucosamide synthesis.

Compared to the molar of diethanolamine used, the temperature variable shows a more significant effect in increasing the amides yield. This is shown in the surface response and contour plots in Figure 4, where the amount of diethanolamine is less than 2.5 molar, the reaction temperature, both at the lower limit of 45ºC and 55ºC, will all result in an amide yield of more than 80%. An interesting result can be seen in the contour plot. If the maximum diethanolamine (4 M) is used and the maximum temperature (55ºC), the yield of fatty acid amides is the minimum value, which is less than 50%.

Overall substrate solubility appears to increase with increasing reaction temperature. Higher temperatures have reducing mass transfer limitations and make the substrate more available for enzymes [7, 19]. Figure 4 shows that the percentage yield reaches a maximum of 97.98% at 55ºC, although in general, the amide yield will decrease slightly if the temperature increases from 50 to 55ºC.

This is thought to be because the lipase enzyme is deactivated at this critical temperature. The synthesis temperature of this fatty acid amide was chosen below 55ºC to prevent damage to the tertiary enzyme structure and reduce the enzyme catalytic activity. For economic reasons as well as enzyme stability, the condensation reaction is stopped after 12 hours. Figures 2,3 and 4 show that the maximum yield surface response of diethanolamide is obtained at the number of enzymes 11-12%, the amount of diethanolamine 2-2.5 molar, and temperature 45-49ºC.

Response of Yield to the Synthesis of Glucosamine

At this stage, each response's optimization objectives are also determined in the Design Expert 10 program. Optimization is carried out following variable data, and measurement results of glucosamide yield data are given in Table-3 below. It was found that the yield response range for glucosamide was 76% to 100%. The relationship between the amide yield response and the independent variables of the amount of enzyme, molar of glucosamine, and temperature, is shown in the following equation:

$$Yield = +88.49 + 6.78A - 25.08B + 0.47C + 1.10AB - 0.12AC + 0.35BC - 0.18A^2 + 0.14B^2 - 3.40C^2 \quad ...(2)$$

where A is the amount of enzyme (%), B is the molar of glucosamine (M), and C is the temperature (ºC). The model above shows that the glucosamide yield response will increase in proportion to the increase in the amount of enzyme and temperature, which is indicated by a positive constant value.

The results of variance analysis for the quadratic model of glucosamide yield are shown in Table-4. It was found that the three variables did not significantly affect the yield of fatty acid amides, indicated by a Prob> F value greater than 0.05. Statistically, this means that these variables only have a negligible effect on the yield of glucosamide.

An additional requirement of whether a model is significant is that it is not aliased. Therefore, based on the sum of squares value, the highest polynomial model was chosen [20]. The model F-value in Table-4 is 2.21, which implies that it is not significant relative to the noise. There is an 11.63% chance that an F-value this large could occur due to noise. In this optimization, the interaction between B and C is a significant variable affecting yield glucosamide, marked by a value of Prob> F, which is less than 0.05. The study of the residual normality plot in Figure-5 shows that the residuals do not follow the regular line. It can be seen that the data does not spread

commonly, which indicates that the results predicted by the model are not close to the actual results.

Table-3. Experimental design for glucosamine condensation reaction and yield response.

Run	A-Enzyme (%)	B-Glucosamine (Molar)	C-Temperature (°C)	R1-Yield of Glucosamide (%)
1	10	3	60	99.520
2	8	2	50	96.600
3	10	1	40	100.00
4	10	3	40	98.595
5	8	2	66.818	97.235
6	6	3	60	100.00
7	6	1	40	100.00
8	8	2	50	96.795
9	6	1	60	96.180
10	8	0.318	50	98.120
11	11.363	2	50	98.535
12	6	3	40	98.715
13	8	2	50	97.495
14	8	2	33.182	97.485
15	8	2	50	98.795
16	8	2	50	97.610
17	10	1	60	76.980
18	8	2	50	98.020
19	4.636	2	50	93.825
20	8	3.682	50	99.325

Table-4. Results of ANOVA for response surface quadratic model for glucosamide synthesis.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	306.54	9	34.06	2.21	0.1163
A-Enzyme	12.14	1	12.14	0.79	0.3956
B-Glucosamine	44.66	1	44.66	2.90	0.1195
C-Temperature	49.69	1	49.69	3.22	0.1028
AB	38.72	1	38.72	2.51	0.1440
AC	52.84	1	52.84	3.43	0.0938
BC	98.35	1	98.35	6.38	0.0301
A^2	8.26	1	8.26	0.54	0.4808
B^2	0.29	1	0.29	0.019	0.8938
C^2	1.67	1	1.67	0.11	0.7490
Residual	154.13	10	15.41		
Lack of Fit	150.88	5	30.18	46.42	0.0003
Pure Error	3.25	5	0.65		
Cor Total	460.67	19			

The response surface and contour plots in Figure-6 illustrate the interaction between the variable amount of glucosamine and the amount of enzyme on the response to glucosamide yield. It appears that the interaction between the two will affect the value of the glucosamide yield response. At a constant temperature of 50ºC, the response of the surface and contour plots as a whole shows that the fatty acid amide yield has reached more than 94%. The two variables' optimal values for the amount of enzyme and glucosamine molar were at 7-10% and 2-3 molar, respectively. However, the percent yield was slightly reduced when the enzyme was added from 10% to 12%. These results indicate the use of excess enzymes does not play a role in achieving high product yields. This is probably because the substrate limitation and enzyme itself will also cause limited mass transfer [11].

The chemoselectivity of the enzymatic reaction will vary depending on the acid/amine ratio. For the acid/amine ratio where the excess acid is present, most of it will form esters. As much as 100% glucosamine is transformed into 6-O-oleoyl-N-methyl-glucamine. If the ratio is less than 1 (excess amine), only oleoyl-N-methyl glucamide is formed.

These results suggest that it is essential to maintain acid-base conditions, especially if the substrate molecules contain ionic groups. Acid-base conditions determine the ionic sites of both the substrate and the enzyme catalyst. Hence, the efficiency and chemoselectivity of the synthesis are carried out [12].

The condensation reaction was stopped after 24 hours, and the yield was checked after the starting materials were washed with acid and alkaline solutions during product purification. Figure-7 shows the interaction effect of the number of enzymes and the reaction temperature.

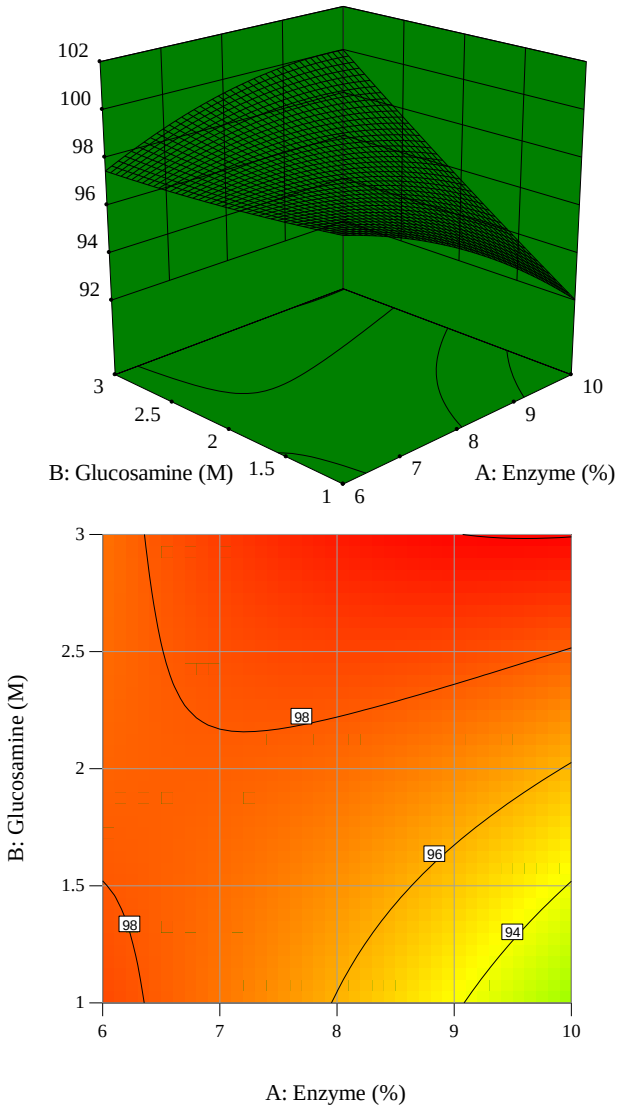


Figure-6. Surface response and interaction contours of the amount of enzyme and molar amine on glucosamide yield.

As with the interaction between enzymes and the amount of glucosamine, the surface response and this contour plot also illustrate a homogeneous result where the whole contour shows a maximum value of> 92% glucosamide yield. The best maximum point is the amount of enzyme of 10%, which is interacted with a reaction temperature of 40ºC. Alternatively, the maximum yield is obtained at selecting the amount of enzyme 6%, but the temperature is at the optimum value of 60ºC.

These results indicate that increasing the temperature does not increase the percent yield due to excess amines. This will inhibit the interaction between the substrate and the enzyme and cause the catalyst's deactivation. The excess substrate will cause enzyme deactivation by dissolving in the microaqueous phase or acting as a competitive inhibitor.

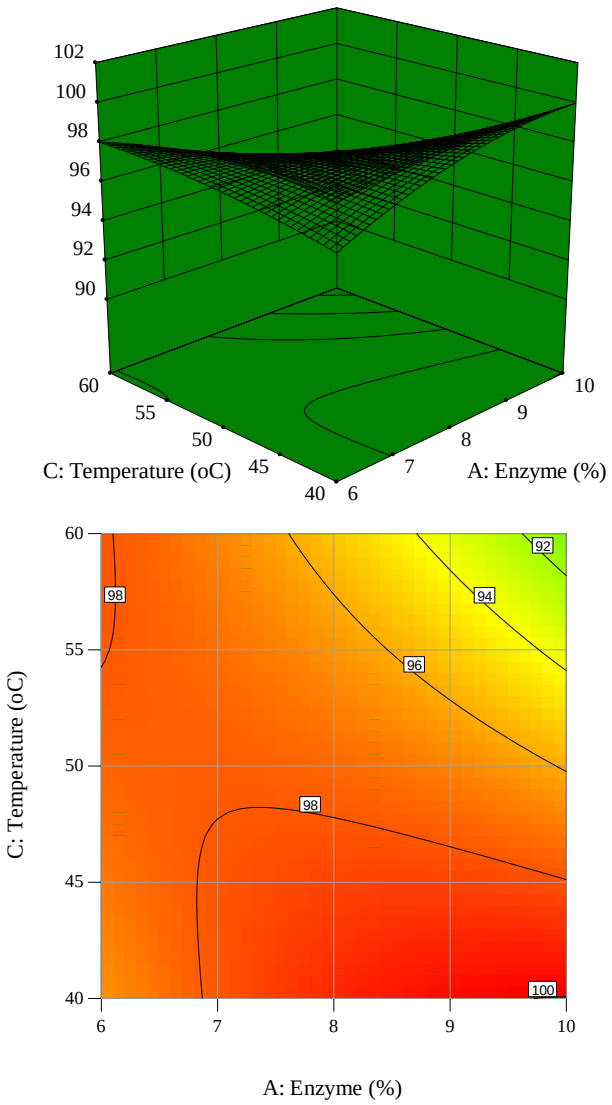


Figure-7. Surface response and interaction contours of temperature and enzyme amount on glucosamide yield.

The results of observing the effect of the amount of added glucosamine (Figure-8) show that the increase in molar amines will significantly increase the yield. However, if a temperature of more than 60°C is used, it is also seen that the increase in concentration will decrease the yield of the product. It seems that the lipase enzyme is less active at temperatures over 60°C, and it is concluded that in this condensation reaction, the activity of the lipase enzyme on the substrate can be triggered by changes in temperature.

Pitzer and Steiner (2016) also investigated changes in product composition throughout the reaction. They found that the reduction in fatty acid methyl esters was in line with amides and esters' formation at the beginning of the reaction. At the start of the reaction, both the amide and the ester have formed, and after 3 hours, the ester formed changes to the amide ester.

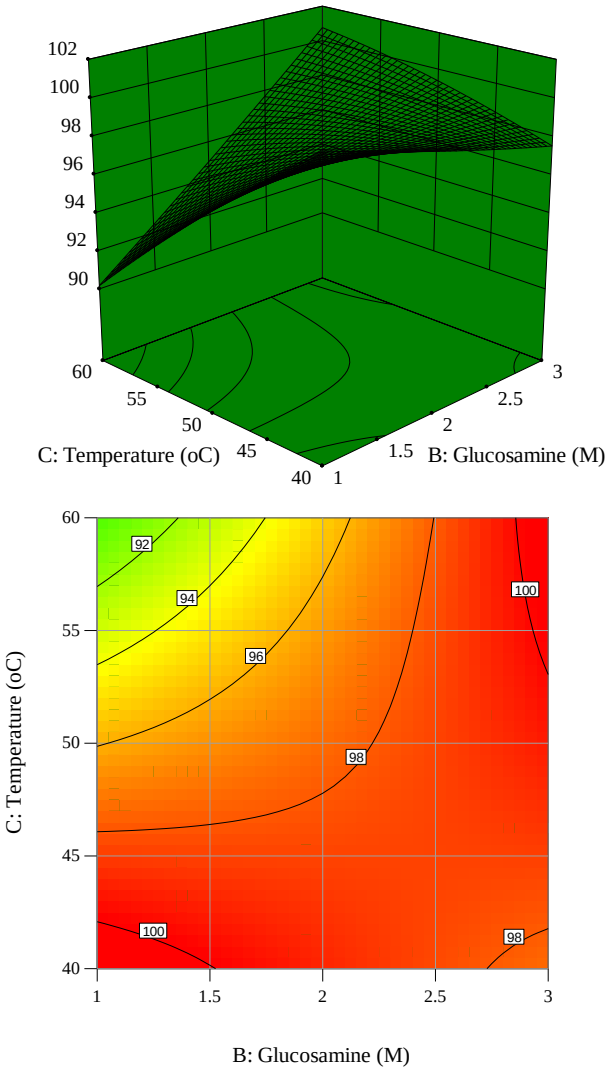


Figure-8. Surface response and interaction contours of temperature and molar amines on glucosamide yield.

At the end of the reaction, the ester formed disappears, and along with it, a new product of 10% is obtained, which is identified as the ester amide, which is probably formed from the ester. After 10 hours of reaction, 100% fatty acid methyl ester will be converted entirely, and the amide yield reaches 80%. The optimum conditions obtained for amide production are at atmospheric pressure, temperature 90°C using the Fatty Acid Methyl Ester: N-methyl-glucamine 1: 1 ratio. Under these conditions, the surfactant mixture thus obtained contains 80% (w: b) amide, 15% ester amide, and 5% N-methyl-glucamine [2]. In this composition, no separation of the mixture is required for industrial raw materials and can be directly used for cosmetic formulations.

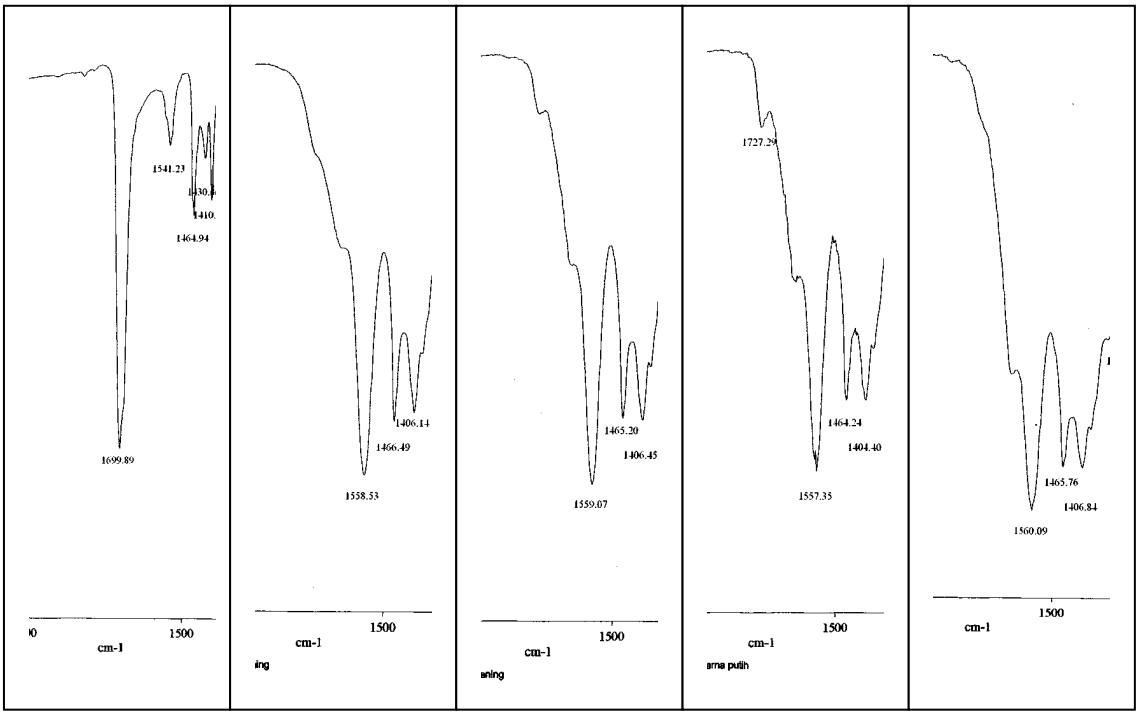
FTIR Spectrum Analysis

Figure-9 shows the FTIR spectrum for the gradual observation of glucosamide synthesis. Analyzes were performed at 0 hours, 24 hours, 45 hours, and 48 hours. Only one carbonyl band was observed when only dodecylic acid was dissolved in tert-amyl alcohol at

1699.89 cm^{-1}. At the start of the synthesis (zero hours), after adding glucosamine to the medium, the carbonyl acid band disappears. A band of 1558.53 cm^{-1} is detected, which indicates the presence of carboxylic ions. The addition of this band indicates that dodecylic acid acts as a phase transfer catalyst for glucosamine. This ion pair is very stable in tert-amyl alcohol (a solvent with a low dielectric constant). The ester derivative will be produced during the initial stage of the reaction, named after the synthesis has been going on for 24 to 45 hours, but will be completely exhausted at the end of the reaction, which is after 48 hours.

CONCLUSIONS

Temperature is one of the crucial factors that influence enzyme activity. In general, product yield will increase with increasing temperature. However, increasing the temperature also inhibits the mobility of the protein segments in the enzyme, wherein the strength of the hydrophobic interactions is reduced. The maximum yield of fatty amides was obtained at temperature 45-49ºC to synthesize dietanolamide and temperature 40-45ºC to synthesize glucosamide. The use of moderate temperature is excellent because it produces a bright color in the final product. The selection of dietanolamine in fatty amide synthesis will produce surfactants with a better degree of polarity because dietanolamine has two hydroxyl groups in its molecules. The optimization results using RSM show that the two models produced can describe the relationship between the three variables to the response, namely fatty acid amide yield.



Gambar 4.48. FTIR spectrum on gradual observation of glucosamide synthesis.at
(a) Pure dodecylic acid ; (b) Reaction time 0 h; (c) 24 h; (d). 45 h and (e) 68 h.

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